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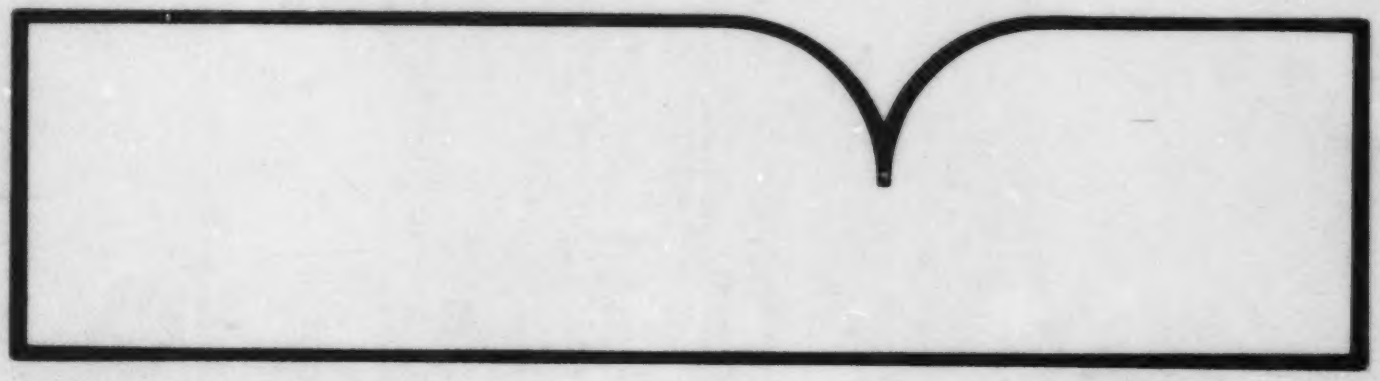

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Chromosome 11 aberrations in small colony L5178Y TK$^{-/-}$ mutants early in their clonal history

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Summary

We have developed a cytogenetic technique that allows observation of chromosome rearrangements associated with TK$^{-/-}$ mutagenesis of the L5178Y/TK$^{+/-}$3.7.2C cell line early in mutant clonal history. For a series of mutagenic treatments we show that the major proportion (93%) of small-colony (σ) mutants studied have chromosome 11 rearrangements (the chromosome containing the thymidine kinase gene) while large-colony (λ) mutants do not have detectable chromosome rearrangements. In addition, we find among the chromosome abnormalities in σ mutants a significant proportion (34%) with dicentric chromosomes involving chromosome 11. These potentially unstable chromosome rearrangements may help to explain the karyotypic instability and heterogeneity among chromosome 11 aberrations previously noted in σ mutants when they are analyzed later in their clonal history.

The L5178Y TK$^{+/-}$ → TK$^{-/-}$ mouse-lymphoma mutagen-assay system, which allows quantitation of forward mutations induced at the autosomal thymidine kinase (TK) gene locus, uses a TK$^{+/-}$ heterozygous cell line, 3.7.2C. TK$^{-/-}$ colonies derived from the TK$^{+/-}$3.7.2C cell line generally display a bimodal frequency distribution of sizes with a small-colony (σTK$^{-/-}$) mutant class and a large-colony (λTK$^{-/-}$) mutant class (Clive et al., 1979).

Several lines of evidence based primarily on genetic features and relative growth patterns (Clive et al., 1979; Moore et al., 1985) have led to the hypothesis that σ and λ mutants result from different extents of genetic damage. In this hypothesis, λ mutants represent events possibly limited to the TK gene locus only, while σ mutants represent effects not only at the TK locus, but at multiple linked loci, rendering the cell less genetically fit and therefore slower growing.

Direct evidence for this view has been obtained by cytogenetic analysis of TK$^{-/-}$ mutants. Banded metaphase chromosome analysis demonstrates a high degree of association of the σ phenotype with the presence of abnormalities involving chromosome 11 (30 of 51 mutants) that persist after 20–30 cell generations (Hozier et al., 1981; Moore et al., 1985), while λ mutants do not have visible chromosome 11 rearrangements. [The thymidine kinase gene has been mapped to chromosome 11 of the mouse by somatic cell genetic techniques (Kozak and Ruddle, 1977)]. However, not all σTK$^{-/-}$ mutants show chromosome 11 abnormalities under these conditions of analysis. Because our previous studies indicated possible karyotype instability in some σTK$^{-/-}$ mutants 20–30 cell

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generations after mutant induction (Hozier et al., 1981), we wished to analyze $TK^{-/-}$ mutants earlier in their clonal history. We developed a technique whereby colonies are removed from soft agar, attached to slides, and prepared for chromosome analysis in situ thus allowing karyotype analysis after 10 or fewer cell generations of clonal growth.

Materials and methods

(A) Cell cultures

L5178Y cell lines and mutant colonies were generated as previously described (Clive and Spector, 1975; Clive et al., 1979; Turner et al., 1984). In order to facilitate early clonal analysis of mutants, colonies were isolated 8 to 10 days after cloning.

(B) Protocol for in situ karyotypic analysis

(1) Preparation of poly-L-lysine (PLL) coated Lab-Tek slides

1 ml of 0.1% w/v PLL (Sigma, degree of polymerization 150 000–300 000 dalton) in distilled water is added to each chamber of a 4-well cell-culture slide (Lab-Tek) and the plastic cover replaced. After incubation at room temperature for 16–24 h, the PLL solution is poured off and all 4 wells rinsed 3 times with distilled water, shaking vigorously between rinses to remove excess water. After the final rinse, the slides are allowed to air dry before replacing the plastic cover. The covered slides are stable for at least 1 month when stored at room temperature.

(2) Isolation of colonies from soft agar

Colonies chosen for analysis are removed from soft agar by aspiration with a pasteur pipet. It is important to obtain the entire colony. The viable cells in the outer shell of the colony are very sensitive to dispersal in the agar while the colony is being culled and great care should be taken to recover as many of these cells as possible.

Colonies are transferred from soft agar to 1.8 ml of F10P medium (Fischer's Medium for Leukemic Cells of Mice with 10% horse serum, 0.2% Pen/Strep (200 000 units penicillin, 200 mg streptomycin), 0.01% w/v sodium pyruvate, and 0.1% w/v Pluronic F68) (Clive and Spector, 1975; Clive et al., 1979; Turner et al., 1984) in a well of a 24-well Linbro plate. One colony is placed in each well using a separate pipet for each colony. The colonies are dispersed by aspirating the medium several times. Plates are incubated at 37°C in a humidified 5% CO_2-in-air incubator for 24–48 h.

(3) Cell attachment and colcemid treatment

PLL-coated 4-chamber Lab-Tek slides are prepared by placing 0.5 ml of F10P in each chamber. The contents of a single well of the Linbro plate are thoroughly dispersed using a 1.0-ml pipet and 0.5–1.0 ml of this cell suspension is transferred to one or more wells of the PLL-treated Lab-Tek slide. Cells that normally do not attach to glass or plastic substrates become firmly attached to such surfaces that have been treated with PLL (Rajendra et al., 1980). We have found this to be the case with L5178Y mouse lymphoma cells. Incubation of the covered slides at 37°C in a humidified 5% CO_2-in-air incubator is continued for 1.5 h. (This allows the cells to attach to the PLL coating.) 10 µl of colcemid (Gibco, 10 µg/ml in Hanks Balanced Salt Solution) is then added to each chamber of the Lab-Tek slide. The contents are mixed with a pasteur pipet and incubated for 30 min at 37°C.

(4) Cell fixation

1 ml of hypotonic solution (0.075 M KCl) is added gently to each slide chamber after removing medium by aspiration or decanting. The transfer should be made quickly to avoid drying of the chambers. Incubation is continued for 8–15 min at room temperature followed by drawing off approximately one-half of the hypotonic solution from each well and replacement with an equal volume of freshly prepared fixative (methanol : acetic acid, 3 : 1). Fixative should be added very gently since this is the most sensitive time for possible detachment and loss of cells. After a short wait (0.5–1 min), each well is filled with additional fixative.

The wells are washed 3 times with fixative by removing most of the old fixative (about 3/4) from each well with a pasteur pipet and then refilling the well with more fresh fixative. Incubation of the slides is continued for 10 min at 37°C

Chambers are then removed from the slides by

pinching at each end while the slide is held in a horizontal plane. (Fixative should not be allowed to drain completely from the surface of the slide.) The remaining rubber gasket material is removed from the slide with forceps. The slide is immediately flooded with fixative using a pasteur pipet. Fixative is then poured off without allowing the slide to dry. The slide is washed with fixative twice more in this way, followed by air drying by vigorous shaking. The quality of the preparation can be monitored by low power phase microscopy.

(c) Staining and analysis of chromosomes

Chromosome banding and analysis of banded karyotypes were performed as previously described (Hozier et al., 1981, 1982). 20 metaphases were examined for each mutant, 10 were photographed and 3 were cut out for detailed karyotyping. To control for possible bias in the choice of chromosomes for analysis, mitotic cells included in the analysis were chosen on the basis of the overall banding patterns rather than for the appearance of any given individual chromosome.

Results and Discussion

We wished to analyze mutants as early in their clonal history as possible so that we might observe the origins of karyotypic changes in $\sigma TK^{-/-}$ mutants. The in situ technique described in the section on Materials and Methods allows us to do such an analysis on each clonal line at about the 10-cell generation stage. We have no way of determining the exact generation stage for a particular small-colony mutant in soft agar, but consideration of colony size and of the division cycle times of σ mutants transferred to suspension culture (Moore et al., 1985) provides us with an estimate of about 10 or fewer division cycles. By comparison, our previous cytogenetic analyses have been performed on colonies that were isolated at a similar stage and grown in suspension culture for an additional 10–20 cycles, or at least a 1000-fold greater level of clonal expansion. Therefore the in situ cytogenetic technique allows us to look very much earlier into the history of particular colonies.

Fig. 1 shows the complete karyotype of a $\sigma TK^{-/-}$ mutant isolated by TFT selection from a hycanthone-treated culture and analyzed by the in situ technique. A dicentric abnormality occurs on the chromosome 11 that we have determined to be the TK-competent chromosome in the $TK^{+/-}$ 3.7.2C cell line by analysis of chromosome heteromorphisms (Hozier et al., 1982). [The other chromosome rearrangements apparent in this karyotype are characteristic of the $TK^{-/-}$ cell line (Hozier et al., 1981).]

We have found that the majority of $\sigma TK^{-/-}$ mutants analyzed in this way show abnormalities in chromosome 11 (see Table 1). In summary, 28 of 30 σ mutants analyzed by the in situ method show chromosome 11 abnormalities 11 of the 30 σ mutants analyzed had cells with a dicentric chromosome 11. In contrast, all 8 λ mutants analyzed in this way show normal chromosomes 11 at the metaphase level of resolution.

Also shown in Table 1 is a summary of results (see Moore et al., 1985, for a complete description) for 51 clonal mutants previously analyzed by suspension culture techniques at the 20–30-cell generation stage (Hozier et al., 1983). Under these conditions, 30 of 51 σ mutants (or 59%) show chromosome 11 abnormalities. Only one of these 51 clonal mutants showed a chromosome 11 dicentric (Moore et al., 1985). None of 17 λ mutants analyzed showed chromosome 11 abnormalities.

The involvement of dicentrics in a significant proportion of $\sigma TK^{-/-}$ mutants analyzed early in their clonal history is potentially important in understanding the origin of chromosomally unstable σ mutants. Fig. 2 shows chromosomes 11 and 17 from a single $\sigma TK^{-/-}$ mutant analyzed at the 10-cell generation stage. There are 3 cells represented (a, b and c), 2 of which (a and b) have rearrangements involving chromosomes 11 and 17. In cell a, a dicentric formed between the TK^{-} chromosome 11 and a chromosome 17 is present. In cell b, only a portion of the chromosome 17 is attached to the distal end of the chromosome 11 resulting most likely in a lethal deficiency. In cell c, an apparently normal set of chromosomes 11 and 17 is present, presumably due to breakage at or near the normal telomeres of the previously joined chromosomes. That is, seen early in the history of this $\sigma TK^{-/-}$ mutant, chromosome rearrangements may be rapidly evolving into near-normal chromosome complements from an initial unstable aberration (i.e., a dicentric chromosome).

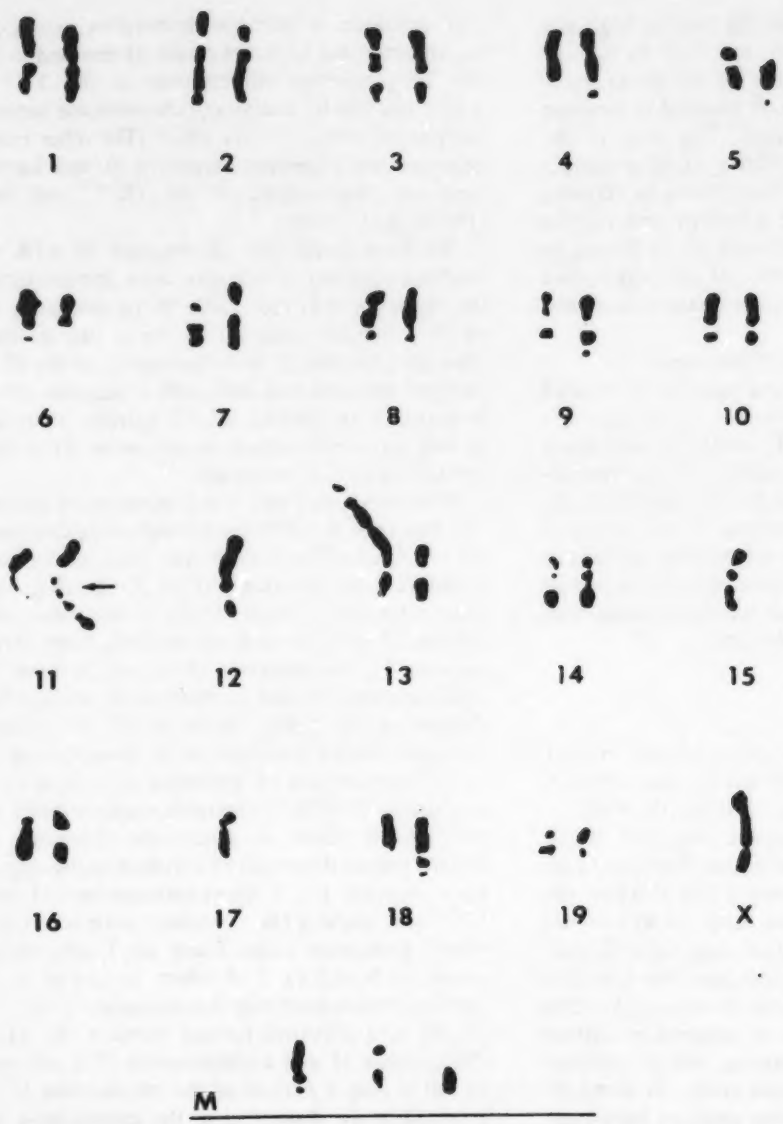

Fig. 1. Karyotype of a hycanthone induced small-colony ($\sigma TK^{-/-}$) mutant prepared by the in situ technique as described in the section on Materials and Methods. A dicentric involving chromosome 11 and chromosome 17 is shown with the site of rearrangement marked with an arrow.

The character of the chromosome rearrangements found in early clonal history provides an explanation for karyotype evolution toward normal chromosome 11 morphology in later clonal history (Hozier et al., 1981; Moore et al., unpublished). That is, the presence of chromosome 11-containing dicentrics in a significant proportion of $\sigma TK^{-/-}$ mutants at the 10-generation stage pro-

TABLE 1

SUMMARY OF CYTOGENETIC ANALYSIS OF $TK^{-/-}$ MUTANTS ANALYSED 20–30 GENERATIONS AFTER CLONING OR LESS THAN 10 GENERATIONS AFTER CLONING

Mutagen	Colony size	Number of $TK^{-/-}$ mutants analysed	Number of mutants with abnormal chromosome 11's (number with dicentrics)	% Mutants with abnormal chromosome 11's (%)
10 generations post-isolation				
Methyl methanesulfonate	λ	1	0	0
	σ	11	11 (6)	100
Hycanthone	σ	10	8 (4)	80
N-Methyl-*N'*-nitro-*N*-nitrosoguanidine	σ	4	4 (1)	100
2-Acetylaminofluorene	λ	5	0	0
	σ	3	3 (0)	100
Benzo[*a*]pyrene	λ	2	0	0
	σ	2	2 (0)	100
Totals, all mutants				
	λ	8	0	0
	σ	30	28 (11)	93
20–30 generations post-isolation				
Ethyl methanesulfonate	λ	4	0	0
	σ	6	3 (0)	50
Methyl methanesulfonate	λ	2	0	0
	σ	7	6 (1)	86
Methyl iodide	λ	1	0	0
Pyrimethamine	λ	10	0	0
	σ	11	6 (0)	55
Hycanthone	σ	10	9 (0)	90
Acyclovir	σ	9	3 (0)	33
Methotrexate	σ	8	3 (0)	37
Totals, all mutagens				
	λ	17	0	0
	σ	51	30 (1)	59

vides a source of unstable chromosome rearrangements that may be resolved, by breakage between centromeres, into the entire range of abnormal, near-normal and normal chromosomes 11 seen in a single clonal cell line at the 20–30-cell generation stage. Karyotypically unstable $\sigma TK^{-/-}$ mutants and at least some of the apparently normal karyotypes in $\sigma TK^{-/-}$ mutants may originate from early unstable (dicentric) rearrangements of chromosome 11. (This does not rule out the possibility that some of the chromosomally 'normal' $\sigma TK^{-/-}$ mutants may contain minor chromosomal rearrangements not detected by standard metaphase chromosome banding techniques and that some of the σ mutants have karyotypically normal chromosomes 11 but may be rendered slow grow-

Fig. 2. Pairs of chromosomes 11 and 17 from three cells of a single hycanthone-induced $\sigma TK^{-/-}$ mutant colony prepared by the in situ technique. Cell (a) has a complete chromosome 17 (shown in brackets) attached to a chromosome 11 in a dicentric configuration involving telomere-to-telomere fusion. Cell (b) has only a portion of the chromosome 17 attached (also shown in brackets). Cell (c) has apparently normal chromosomes 11 and 17.

ing by rearrangements on the other chromosomes.) It is also possible that the high proportion of σ mutants with chromosome 11 abnormalities observed by in situ analysis may reflect mutagen specificities in the ability to produce large chromosome damage events (Krehl et al., 1973; Clive et al., 1984), though all mutagens in this series showed a high proportion of σ mutants with chromosome 11 damage. All of these results are consistent with the previously established chromosomal nature of mutagenic events at the TK locus in $\sigma TK^{-/-}$ mutants (Hozier et al., 1981, 1982).

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References

Clive, D., and J.F.S. Spector (1975) Laboratory procedure for assessing specific locus mutations at the TK locus in cultures L5178Y mouse lymphoma cells. Mutation Res., 31, 17–29.

Clive, D., K.O. Johnson, J.F.S. Spector, A.G. Batson and M.M.M. Brown (1979) Validation and characterization of the L5178Y/$TK^{+/-}$ mouse lymphoma assay system. Mutation Res., 59, 61–108.

Clive, D., R. Krehl, N.T. Turner, J. Hozier, J. Sawyer (1984) Types of cytogenetic damage detected in the L5178Y/$TK^{+/-} \rightarrow TK^{-/-}$ mouse lymphoma assay by banded karyotype analysis. Environ. Mutagen Soc., 15, 57 (abstract).

Hozier, J., J. Sawyer, M. Moore, B. Howard and D. Clive (1981) Cytogenetic analysis of the L5178Y/$TK^{+/-} \rightarrow TK^{-/-}$ mouse lymphoma mutagenesis assay system. Mutation Res., 84, 169–181.

Hozier, J., J. Sawyer, D. Clive and M. Moore (1982) Cytogenetic distinction between the TK^{+} and TK^{-} chromosomes in the L5178Y $TK^{+/-}$ 3.7.2C mouse-lymphoma cell line. Mutation Res., 105, 451–456.

Hozier, J., J. Sawyer, D. Clive and M.M. Moore (1983) Cytogenetic analysis of small-colony L5178Y $TK^{-/-}$ mutants early in their clonal history. Ann. N.Y. Acad. Sci., 407, 423–425.

Kozak, C.A., and F.H. Ruddle (1977) Assignment of the genes for thymidine kinase and galactokinase to *Mus musculus* chromosome 11 and the preferential segregation of this chromosome in Chinese hamster/mouse somatic cell hybrids. Somatic Cell Genet., 3, 121–133.

Krehl, R., D. Clive, J. Hozier and J. Sawyer (1983) Types of cytogenetic damage detected in the L5178Y/$TK^{+/-} \rightarrow TK^{-/-}$ mouse lymphoma assay by banded karyotype analysis. Genotox. Environ. Mutagen Soc., 1, 20 (abstract).

Moore, M.M., D. Clive, J.C. Hozier, B.E. Howard, A.G. Batson, N.T. Turner and J. Sawyer (1985) Analysis of trifluorothymidine resistant (TFT^{r}) mutants of L5178Y/$TK^{+/-}$ mouse lymphoma cells. Mutation Res., 151, 161–174.

Rajendra, B.R., L.J. Sciorra and M.L. Lee (1980) A new and simple technique for chromosomal preparations from peripheral blood lymphocytes, amniotic cell-cultures, skin fibroblasts, bone marrow and single cell clones when the yields from harvests are low. Human Genet., 55, 363–366.

Turner, N.T., A.G. Batson and D. Clive (1984) Procedures for the L5178Y/$TK^{+/-} \rightarrow TK^{-/-}$ mouse lymphoma cell mutagen assay, in: B. Kilbey, M. Legator, W. Nichols and C. Ramel (Eds), Handbook of Mutagenicity Test Procedures, 2nd edn., Elsevier, Amsterdam, pp. 239–268.